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(57) Abstract

Peptides and a diagnostic antigen containing one of said peptides and having the ability to detect antibodies against at least one type of picornavirus and flavivirus are described. The use of said diagnostic antigen for diagnosing infections caused by picornavirus and/or flavivirus and for discriminating between false and true diagnosed HIV-1 p17 positive sera is also described, as well as vaccines against diseases caused by picornaviruses and/or flaviviruses.

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ENTEROVIRUS PEPTIDES

The present invention relates to new peptides, to a diagnostic antigen, to the use of said diagnostic antigen in immunoassays and to a vaccine containing said diagnostic antigen.

Background art

Picornaviruses are a family of small RNA viruses which are responsible for a variety of diseases in humans and animals. The diseases may be severe, like poliomyelitis (caused by polioviruses types 1, 2, and 3), hepatitis (caused by hepatitis A virus) and generalized neonatal enterovirus infection (often caused by Coxsackie and ECHO viruses), as well as mild, like the common cold (caused by rhinoviruses). Picornaviruses then are significant pathogens, and the diagnosis of infections caused by them is an important medical task. A clinical virological laboratory receives many samples every year for diagnosis of picornavirus infections. The picornavirus family is divided into the enterovirus, rhinovirus and hepatitis A subfamilies. Among these, the enteroviruses and hepatitis A viruses are the ones where a diagnosis is most often sought. The enteroviruses are further divided into polio-, Coxsackie A, Coxsackie B and ECHO viruses on the basis of their pathogenicity in mice. The enteroviral disease where a diagnosis is most often required is aseptic meningitis, but myocarditis, encephalitis, generalized neonatal infection and hand-, foot and mouth disease also constitute rather frequent clinical problems.

Flavivirus is another virus family which e.g. causes yellow fever and encephalitis. These diseases are spread by mosquitos and ticks and constitute a major worldwide problem. The Dengueviruses and TBE-virus are types of flaviviruses.

There is currently no therapy for these infections, so the diagnosis of picornavirus and flavivirus infections serves mostly to exclude other diseases or to give epidemiological information.

The diagnosis of a picornavirus infection can be made either by virus isolation or by serology. In the latter case, for enteroviruses, a serum sample taken early in the course of the infection (an acute phase serum) and a serum taken 1-3 weeks later (a convalescent phase serum) are analyzed with an antibody assay, most commonly a complement fixation assay (see e.g. Sever JL. Application of a microtechnique to viral serological investigations. J. Immunol, 88:320-329). The antigen then is a purified or semipurified preparation from picornavirus-infected cells. If non-heat-treated virus antigens are used, a relatively type-specific antibody assay is obtained. If a heat-treated viral antigen is used, a more group-specific assay is obtained. Presumably, the virus undergoes a conformational change upon heating, exposing group-specific antigenic determinants. The conformational change mostly seems to involve the N-terminus of VP1 [Fricks CE, Hogle JM. Cell-induced conformational change in poliovirus: Externalization of the amino terminus of VP1 is responsible for liposome binding. J. Virol. 64:193-1945 (1990)]. There are some indications that antibodies to this portion of VP1 can influence the course of infection [Chow M, Yabrov R, Bittle J, Hogle JM, Baltimore D. Synthetic peptides from four separate regions of the poliovirus capsid protein VP1 induce neutralizing antibodies. Proc Natl Acad Sci USA 82:910-914 (1985)], this making it interesting also as a candidate for vaccination purposes. For flaviviruses, a variety of serological techniques exist, all of which however have great limitations due to lack of sensitivity or specificity.

Thus, there is a need for a means for a more exact diagnosis of diseases caused by picornavirus and flavi-

virus, which could further specify and facilitate the treatment of the disease concerned.

Further, there is a need for vaccines for different diseases caused by picornavirus and flavivirus.

One object of the present invention is to provide new peptides which can be used as a diagnostic antigen in immunoassays.

Another object of the present invention is to use a diagnostic antigen for more specific diagnosing of diseases or infections caused by picornavirus and/or flavivirus and/or discriminating between false and true diagnosed HIV-1 positive sera.

Still another object of the present invention is to provide a vaccine based on said peptides against diseases or infections caused by picornavirus and/or flavivirus.

All these objects are achieved by the present invention.

Description of the invention

In one aspect, the present invention relates to peptides of the formula:

a) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-His-Thr-Ser-Gln-Val-R^2-X-Z, wherein Y^1 is Ala or Val,

b) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-Ala-Thr-Asn-Pro-Leu-R^2-X-Z, wherein Y^1 is Ala or Val,

c) H-X-R^1-Ala-Ala-Glu-Thr-Gly-His-Thr-Ser-Y^1-Val-R^2-X-Z, wherein Y^1 is Ser or Asn,

d) H-X-R^1-Y^1-Asp-Thr-Gly-His-Gly-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ala or Thr and Y^2 is Val or Ile,

e) H-X-R^1-Thr-Asp-Y^1-Gly-His-Asp-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ser or Thr and Y^2 is Ile or Val,

and

f) H-X-R^1-Ala-Glu-Thr-Gln-Y^1-Gly-Thr-Y^2-Val-R^2-X-Z, wherein Y^1 is His or Tyr and Y^2 is Ile or Thr,

in which one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating amino acid sequence, R^1 and R^2 represent optional amino acid residues, wherein R^1 and R^2 together represent at

most 25 amino acid residues, and Z represents $-NH_2$ or -OH.

In another aspect, the present invention relates to a diagnostic antigen having the ability to detect antibodies against at least one type of picornavirus and flavivirus in sera, and/or having the ability of binding to antibodies with a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or cluster of epitopes of HIV-1 p17, wherein said antigen mainly comprises an antigen selected from the peptides according to claim 1 and antigenic parts thereof.

In still another aspect, the present invention relates to the use of at least one diagnostic antigen for diagnosis of infections caused by picornaviruses and/or flaviviruses, wherein the diagnostic antigen or antigens having the ability to detect antibodies against at least one type of picornavirus and flavivirus are added to a serum from a subject suspected to have a recent or previous picornavirus and/or flavivirus infection, and possible antigen/ antibody complexes formed are detected using an immunoassay, the diagnosis of said recent or previous infection(s) caused by picornaviruses and/or flaviviruses being established based either upon demonstration of antibodies in a single serum or upon a comparison of the amounts of antibodies detected in both acute and convalescent phase sera, and if said amount in the convalescent phase serum is significantly larger than in the acute phase serum, the subject donating the serum suffers or has suffered from a picorna- and/or flavivirus infection, wherein the antigen containing the peptides

a) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-His-Thr-Ser-Gln-Val-R^2-X-Z, wherein Y^1 is Ala or Val, is primarily used for diagnosis of diseases caused by Coxsackievirus,

b) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-Ala-Thr-Asn-Pro-Leu-R^2-X-Z, wherein Y^1 is Ala or Val, is primarily used for diagnosis of diseases caused by poliovirus,

c) H-X-R^1-Ala-Ala-Glu-Thr-Gly-His-Thr-Ser-Y^1-Val-R^2-X-Z, wherein Y^1 is Ser or Asn, is primarily used for

diagnosis of deseases caused by rhinovirus,

d) H-X-R^1-Y^1-Asp-Thr-Gly-His-Gly-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ala or Thr and Y^2 is Val or Ile, is primarily used for diagnosis of diseases caused by yellow fever virus and Japanese encephalitis virus,

e) H-X-R^1-Thr-Asp-Y^1-Gly-His-Asp-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ser or Thr and Y^2 is Ile or Val, is primarily used for diagnosis of diseases caused by TBE-virus,

and

f) H-X-R^1-Ala-Glu-Thr-Gln-Y^1-Gly-Thr-Y^2-Val-R^2-X-Z, wherein Y^1 is His or Tyr and Y^2 is Ile or Thr, is primarily used for diagnosis of diseases caused by Dengue- and rhinovirus,

in which one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating amino acid sequence, R^1 and R^2 represent optional amino acid residues, wherein R^1 and R^2 together represent at most 25 amino acid residues, and Z represents $-NH_2$ or -OH.

In still another aspect, the present invention relates to the use of a diagnostic antigen for discriminating between a false and true diagnosed HIV-1 positive serum sample, which has first been diagnosed as positive in a standard HIV-1 antibody screening EIA test and then found to exhibit a p17 pattern of serological activity in an electrophoretic immunoblot assay, wherein at least one diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or cluster of epitopes of HIV-1 p17, optionally coupled to a carrier, is added to said serum sample, and possible antigen/antibody complexes formed are detected using an immunoassay, the discrimination being established such that said serum sample is false HIV-1 positive if said complexes are detected, and true HIV-1 positive if said complexes are not detected.

In still another aspect, the present invention relates to a vaccine composition which comprises as an immunizing component at least one picornavirus and/or flavivirus antigen selected from at least one of the peptides above.

Other characteristics and features of the present invention appear from the attached claims.

In the peptides according to the present invention, one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating sequence of at least 4, and preferably 8, particular amino acid residues, which are all chosen from the group consisting of -Thr- and -Ser-. When X is an amino acid sequence, it can be located either in the C- or N-terminus of the peptides but not in both ends at the same time. If it is for example located in the C-terminus, X in the N-terminus corresponds to a chemical bond and vice versa. However, X can be a bond at both ends of the peptide according to the present invention. The amino acid sequence acts as a coupling-facilitating spacer, which permits proper binding to the carrier to which the peptides according to the present invention will be bound during the discrimination method. The sequence X should not be an amino acid sequence which adversely affects the result of the diagnosis method. Accordingly, it should not have too high a charge or be too hydrophobic and it should not disturb the conformation of the peptides. The amino acids threonine and serine also fulfill these requirements particularily well, and any one of the amino acids in said sequence X can be threonine or serine. The number of amino acids in this spacer sequence should be at least 4, but in a preferred embodiment according to the present invention 8 amino acids are used.

R^1 and R^2 are optional amino acid residues and together represent at most 25 amino acid residues, preferably 20. Further, this means that one or both of R^1 and R^2 can be a chemical bond, i.e. contain no amino acids. If R^1

is a sequence containing e.g. 25 amino acid residues, R^2 must be a chemical bond and vice versa.

The expression "and antigenic parts thereof" as used herein means antigenic parts in the essential amino acid sequence between R^1 and R^2 in the peptides according to the present invention.

The polypeptides according to the present invention can be bound via the amino acid sequence X to a carrier by physical/chemical interaction, as for example covalent binding, ionic binding, hydrogen binding or hydrophobic binding. Examples of covalent binding are ester, ether and disulfide binding.

The expression "carrier" as used herein should be interpreted broadly, and it may be any material which is compatible with and not adversely affects the method according to the present invention, for example resins, microplates, plastic surfaces, gels, matrixes etc.

The expression "epitope" as used herein means antigenic or immunogenic determinant and relates to a specific part of a structure of an antigen inducing an immuno response, and the antibodies produced are directed against this part.

The immunoassay method according to the present invention can be an enzyme immunoassay (EIA), radioimmunoassay (RIA), immunoassay involving metal labelling, fluorescence immunoassay (FIA) or an immunoassay in which the peptide is soluble and inhibits another reaction.

The vaccine composition according to the present invention comprises at least one picornavirus and/or flavivirus antigen selected from the peptides according to the present invention together with a non-toxic pharmaceutically acceptable carrier and/or a diluent in an amount effective to protect a subject from diseases caused by picornavirus and/or flavivirus.

Further, the vaccine composition comprises an antigen adjuvant in an amount which together with an amount of said picornavirus and/or flavivirus antigen(s) is effec-

tive to protect a subject from diseases caused by picornaviruses and/or flaviviruses.

Further, the vaccine composition additionally comprises one or more buffers and/or preservatives.

The peptides according to the invention are derived from sequences which are exposed upon heat inactivation of picornavirus or are situated on the other envelope proteins of flavivirus particles, and they are able to detect broadly cross-reactive picornavirus and flavivirus antibodies. This makes it possible to diagnose picornaviral and/or flaviviral infections or diseases by the use of only one or a few peptides from the highly conserved antigenically active picornaviral and flaviviral sequence.

The location of this sequence on the envelope protein, its pronounced evolutionary conservation in flaviviruses and the proven antigenicity of analogous sequences in picornaviruses make this sequence especially accessible for antibodies suitable for diagnostic and immunisation purposes.

The peptide sequences according to the present invention can be said to derive from a common basic structure which can be expressed as the consensus sequence aaDTGHxLxV where the capital letters relate to amino acids of strong or complete conservation, the small letters relate to amino acids present in more than half of the cases, and x relates to any amino acid. This common basic structure has been discovered on one and the same occasion and has been described in an article by Blomberg & Medstrand in New Biologist 2:1044-1046 (1990). In picornavirus, this sequence is situated at one site in VP1 in the amino acids 20-40. In flavivirus, it is situated about 80 amino acids from the transmembrane region of the E-glucoprotein. In both cases, the sequence participates in interactions with the outer membrane of the host cell. In both cases, the sequence is also concealed to antibodies during part of the replication cycle of the virus, but becomes accessible to them, for instance when the

virus has come into contact with the outer membrane of the cell. Thus, the peptides according to the present invention can be considered to belong to a previously unknown class of sequences having a common structure and function. Although the peptides can be used for diagnosing diseases caused by both picornavirus and flavivirus, the peptides can, because of their common origin, structure and function, however with a certain, appropriate variation of the amino acids, be comprised by the same inventive concept. Many or all of the peptides, preferably 3-4, according to the present invention can be included in one and the same assay for diagnosing different picornavirus- and flavivirus-induced diseases.

As to the presence of the peptides according to the present invention in vaccine compositions, it may initially be stated that the above-mentioned natural basic sequence is included in a region where it has earlier been demonstrated that peptides give rise to neutralising antibodies in rabbit immunisation. In these experiments, however, the peptides according to the present invention were not used, but yet closely related peptide sequences. The evolutionary conservation of said basic sequence confers e.g. the advantage of producing a vaccine inhibiting infection involving many different cold viruses.

As to peptides deriving from flavivirus, the inventor, together with Dr Vincent Deubel at the Arbovirus laboratory at the Pasteur Institute in Paris, has tested the ability of previously known monoclonal antibodies having a virus-neutralising effect, of binding to the peptides according to the present invention. One of them, namely peptide (f) directed against Denguevirus, was found, for instance, to strongly bind to a peptide containing the Dengue variant of the common basic structure. Since the ability of producing neutralising antibodies is one of the characteristics required of a vaccine, it must be considered highly likely that the peptides according to the present invention are usable as

antigens in vaccine compositions against the above-mentioned picornavirus- and/or flavivirus-induced diseases.

Table 1 refers to the alignment of some different viral amino acid sequences from the region of similarity with HIV-1 p17.

Table. Alignment of picorna- (VP1), flavi-(E protein) and coronaviral (VP1) amino acid sequences from the region of similarity with HIV-1 p17.

HIV-1 3b clone hxb2		LLLDTGH SNQVSQNY	
mn		LLLDTGnrgnSsQVSQNYpivqnlqg	
rf		LLLDTGn gsQVSQNY	
mal		LLLAQQLLAsTkn SssVSQNY	
HIV-1 consensus		LLLDTGH SNQVSQNY	
		L K SS	
Less common variants		gak g k	
		s n	
Picornaviruses			position in polyprotein
Coxsackie B1 VP1	vsskptnsesipaltAAETGH	TSQVvpsdtnqtrhv	603-612 ⟶
Swine vesicular disease VP1	igsgpvnsesipaltAAETGH	TSQVvpsdtnqtrh	601-610
Coxsackie B3 VP1	vgtgpnnseaipaltAAETGH	TSQVvpgdtnqtrh	603-612
Coxsackie B4 VP1	iargpsnseqipaltAvETGH	TSQVdpsdtnqtrh	601-610
Poliovirus 1 strain Mahoney	teasgpthskeipaltAvETGa	TNplvpsdtvqtrh	624-633 ⟶
duplication	psdtvg	trhv	
Human rhinovirus 1B VP1	ikeshhttsnsapl1dAAETGH	TSNVqpedaietry	606-615 ⟶
Human rhinovirus 2 VP1	inssnpttsnsapaldAAETGH	TSSVqpedvietry	603-612
duplication	pedvie	try	
Foot-and-Mouth dis v VP1	AsDTae	TTNV	676-685
Flaviviruses			
Yellow fever virus	tsykictdknffvknptDTGH	gTvVngkvskgapc	595-604
Japanese encephalitis virus	ttygnctekfsfaknpADTGH	gTvVielsysqsdg	537-545
St Louis encephalitis virus	ttygnodsaftfsknptDTGH	gTvIvelgytgsng	603-612
Tick-borne encephalitis v	ptdsgh	dtvv	598-607
Dengue 2J	AETqH	gTIV	593-601
Dengue 4	AETqh	gTtV	592-600

The peptides according to the present invention can be used as a diagnostic antigen to detect antibodies in both acute and convalescent phase sera. If the amount of antibody detected in the convalescent serum is significantly larger than in the acute serum, the person donating the blood samples is believed to suffer from a picornavirus infection. The expression "significantly larger" as used herein is a standard concept in this area (see J. Blomberg, I. Nilsson and M. Andersson. 1983. Viral antibody screening system that uses a standardized single dilution immunoglobulin G enzyme immunoassay with multiple antigens. J. Clin. Microbiol. 17:1081-1091.)

If antibodies belonging to the IgM class and reactive with at least one of the picornaviral and/or flaviviral peptides described in the present invention are detected in one or more sera from a patient, then the patient is likely to suffer from a disease caused by a picornavirus and/or a flavivirus.

The peptides according to the present invention can further be used as a supplementary test during the investigation of an antibody reaction with HIV-1 p17. If the anti-p17 reaction can be abrogated by preabsorption or competition with one of the peptides according to the present invention, it is unlikely that the anti-p17 reaction is caused by antibodies to a picornavirus and/or flavivirus.

The as yet unpublished Swedish patent application 8903985-3, filed on November 27, 1989, also by Replico AB, describes new peptides, diagnostic antigens containing said peptides and having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or a cluster of epitopes of HIV-1 p17, and a method of discriminating between a false and true diagnosed HIV-1 positive serum sample using an immunoassay. The technical background, proper definitions, and some of the methods used in connection with the use of the peptides according

to the present invention in the p17 area are described in said Swedish patent application.

After the introduction of large-scale HIV-1 antibody testing, it has been observed that a small portion of sera from humans unlikely to be HIV-1 infected contains antibodies to one or some HIV-1 proteins, mostly derived from the gag part of the molecule. Such reactivities may give rise to HIV-1 electrophoretic immunoblot patterns which are hard to interpret. One of the most common types of such HIV-1 EIB reactivities is characterized by a reactivity with HIV-1 p17, but with no other HIV-1 protein. The reactivity with p17 peptides in sera from healthy Swedes which had the p17 pattern of serological reactivity has been studied. By direct binding and absorption experiments it has been shown that, in contrast to EIB confirmed HIV-1 antibody positive sera, the p17 positive sera reacted with peptides from the C-terminus of p17. It has furthermore been shown that they contain a stretch highly similar to a conserved sequence from the N-terminus of VP1 of some enteroviruses, and to a lesser extent, flaviviruses.

Brief description of the drawings

Figure 1 shows absorption of five p17 positive sera with resin without peptide, resin with HIV-1 gag 118-140, resin with HTLV-I gag 111-130 (the C-terminus of HTLV-I), and resin with Coxsackie B1 VP1 25-53. The intensity of the p17 band in EIB (y axis) was measured with a densitometer.

Figure 2 shows the serological reactivity of a peptide derived from Coxsackie B1 VP1 25-53 with

a/ acute and convalescent phase sera from five cases of aseptic meningitis with significant Coxsackie CF titre rises,

b/ 10 p17 reactive sera,

c/ 19 HIV seronegative blood donors and

d/ 6 confirmed HIV-1 antibody positive sera.

Experiment with a Coxsackie B1 VP1 derived peptide

A peptide from amino acids 25-53 of the Coxsackie B1 VP1 sequence (Iizuka, N., S. Kuge and A. Nomoto. 1987. Complete nucleotide sequence of the genome of Coxsackievirus B1. Virology 156:64-73.) was synthesized, and its ability to absorb p17 antibodies was compared with that of HIV-1 hxb2 gag 118-140 (Fig. 1). In five out of five sera tested, this peptide in resin-bound form absorbed out all p17 EIB reactivity. HIV-1 hxb2 gag 118-140 also absorbed these antibodies, but less completely. Absorption with resin alone, or with HTLV-I gag 111-130 did not affect the p17 EIB reactivity. The p17 reactive antibodies in these sera thus must bind to epitopes simulated by the resin-bound Coxsackie-derived peptide. The peptide was present in vast molar excess compared to antibodies, thus favouring absorption also of low-affinity antibodies.

The reactivity of p17-derived peptides was compared with that of a Coxsackie B1-derived peptide. 10 p17 positive sera, 19 HIV seronegative blood donors, 6 sera from confirmed HIV-1 seropositive persons (1 adult and 5 children, aged 1-7), and acute and convalescent sera from six patients with aseptic meningitis with significant (at least fourfold) titre rises in complement fixation antibody tests against Coxsackie B1-B6 antigen were analyzed.

As to antibody activity against the Coxsackie peptide among the p17 reactive sera diluted 1/200, it was found that they did not react significantly more than blood donor sera (Fig 2). This may indicate that the Coxsackie peptide, when absorbed to plastic, does not assume a conformation suitable for binding of p17 reactive antibodies, or that it can bind many other antibodies, which obscures a possible correlation. Six HIV-1 confirmed positive sera with clear p17 bands reacted weakly. Five of the sera were selected from children, to reduce the likelihood of previous exposure to a Coxsackie-like virus. Thus, there was no cross-reactivity of p17 antibodies arising during HIV-1 infection to the Coxsackie peptide.

Finally, six serum pairs were tested from patients with aseptic meningitis who had significant titre rises in the complement fixation test with Coxsackie B1-B6 antigen. It was found that five exhibited clear rises in absorbance difference and one reacted strongly on both sera (Fig. 2). A CF antibody titre rise in this system indicates an enterovirus infection, not necessarily with Coxsackie B1. When the same sera were tested at a dilution of 1/50, almost all reacted strongly (not shown), attesting to the high antigenicity and frequent recognition of this peptide sequence. The Coxsackie peptide may then be a group-reactive reagent suitable for serological diagnosis of enterovirus infections. The cross-reaction between the Coxsackie B1 VP1 peptide and HIV-1 hxb2 p17 thus was unidirectional, from the enterovirus to HIV-1, but not vice versa. Antibody reactions with the Coxsackie peptide were very common in Swedish sera diluted 1/50. Only a few of them can bind also to HIV-1 p17 in EIB.

The immunoassays used are described in the Swedish patent application above. Coxsackie antibodies were measured by titration in standard microtitre complement fixation antibody tests with Coxsackie B1, B2, B3, B4, B5, and B6 antigens.

The diagnostic antigens according to the present invention can be utilized in methods for the same purpose as the claimed use of said diagnostic antigens.

CLAIMS

1. Peptides of the formula

a) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-His-Thr-Ser-Gln-Val-R^2-X-Z, wherein Y^1 is Ala or Val,

b) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-Ala-Thr-Asn-Pro-Leu-R^2-X-Z, wherein Y^1 is Ala or Val,

c) H-X-R^1-Ala-Ala-Glu-Thr-Gly-His-Thr-Ser-Y^1-Val-R^2-X-Z, wherein Y^1 is Ser or Asn,

d) H-X-R^1-Y^1-Asp-Thr-Gly-His-Gly-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ala or Thr and Y^2 is Val or Ile,

e) H-X-R^1-Thr-Asp-Y^1-Gly-His-Asp-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ser or Thr and Y^2 is Ile or Val,

and

f) H-X-R^1-Ala-Glu-Thr-Gln-Y^1-Gly-Thr-Y^2-Val-R^2-X-Z, wherein Y^1 is His or Tyr and Y^2 is Ile or Thr,

in which one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating amino acid sequence, R^1 and R^2 represent optional amino acid residues, wherein R^1 and R^2 together represent at most 25 amino acid residues, and Z represents $-NH_2$ or -OH.

2. Peptides according to claim 1, wherein the coupling-facilitating amino acid sequence represents at least 4, preferably 8, amino acid residues, which are each selected from the group consisting of -Thr- and -Ser-, and R^1 and R^2 together preferably represent at most 20 amino acid residues.

3. A diagnostic antigen having the ability to detect antibodies against at least one type of picornavirus and flavivirus in sera, and/or having the ability of binding to antibodies with a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or cluster of epitopes of HIV-1 p17, c h a r a c t e r -

i s e d in that it mainly comprises an antigen selected from the peptides according to claim 1 and antigenic parts thereof.

4. Use of a diagnostic antigen according to claim 3 for the diagnosis of infections caused by picornaviruses and/or flaviviruses, wherein a diagnostic antigen having the ability to detect antibodies against at least one type of picornavirus and flavivirus is added to a serum from a subject suspected to have a recent or previous picornavirus and/or flavivirus infection, and possible antigen/antibody complexes formed are detected using an immunoassay, the diagnosis of said recent or previous infection or infections caused by picornavirus and/or flavivirus being established based either upon demonstration of antibodies in a single serum or upon a comparison of the amounts of antibodies detected in both acute and convalescent sera, and if said amount in the convalescent serum is significantly larger than that in the acute serum, the subject donating the serum is likely to suffer from a picorna- and/or flavivirus infection, wherein the antigen containing the peptide

a) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-His-Thr-Ser-Gln-Val-R^2-X-Z, wherein Y^1 is Ala or Val, is primarily used for diagnosis of diseases caused by Coxsackievirus,

b) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-Ala-Thr-Asn-Pro-Leu-R^2-X-Z, wherein Y^1 is Ala or Val, is primarily used for diagnosis of diseases caused by poliovirus,

c) H-X-R^1-Ala-Ala-Glu-Thr-Gly-His-Thr-Ser-Y^1-Val-R^2-X-Z, wherein Y^1 is Ser or Asn, is primarily used for diagnosis of diseases caused by rhinovirus,

d) H-X-R^1-Y^1-Asp-Thr-Gly-His-Gly-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ala or Thr and Y^2 is Val or Ile, is primarily used for diagnosis of

diseases caused by yellow fever virus and Japanese encephalitis virus,

e) H-X-R^1-Thr-Asp-Y^1-Gly-His-Asp-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ser or Thr and Y^2 is Ile or Val, is primarily used for diagnosis of diseases caused by TBE-virus,

and

f) H-X-R^1-Ala-Glu-Thr-Gln-Y^1-Gly-Thr-Y^2-Val-R^2-X-Z, wherein Y^1 is His or Tyr and Y^2 is Ile or Thr, is primarily used for diagnosis of diseases caused by Dengue- and rhinovirus,

in which one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating amino acid sequence, R^1 and R^2 represent optional amino acid residues, wherein R^1 and R^2 together represent at most 25 amino acid residues, and Z represents $-NH_2$ or -OH.

5. Use of a diagnostic antigen according to claim 3 for discriminating between a false and true diagnosed HIV-1 positive serum sample, which has first been diagnosed as positive in a standard HIV-1 antibody screening EIA test and then found to exhibit a p17 pattern of serological activity in an electrophoretic immunoblot assay, wherein at least one diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or cluster of epitopes of HIV-1 p17, optionally coupled to a carrier, is added to said serum sample, and possible antigen/antibody complexes formed are detected using an immunoassay, the discrimination being established such that said serum sample is false HIV-1 positive if said complexes are detected, and true HIV-1 positive if said complexes are not detected.

6. Use according to claims 4 and 5, c h a r a c t e r i s e d in that the immunoassay for diagnosis of diseases caused by picornavirus and/or flavivirus and for detection of said antibody/antigen complex, respectively, is an enzyme immunoassay (EIA), radioimmuno assay (RIA),

immunoassay involving metal labelling, fluorescence immunoassay (FIA) or an immunoassay in which the peptide is soluble and inhibits another reaction.

7. Use according to claims 4 and 5, c h a r a c t e r i s e d in that the carrier is a resin, microplate, plastic surface, gel or matrix.

8. Use according to claims 4-7, c h a r a c t e r i s e d in that in the diagnosis and discrimination use is simultaneously made of many or all, preferably 3-4, of the diagnostic antigens according to claim 3.

9. A vaccine composition, c h a r a c t e r i s e d in that it comprises as an immunizing component, at least one antigen selected from at least one peptide from the group consisting of

a) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-His-Thr-Ser-Gln-Val-R^2-X-Z, wherein Y^1 is Ala or Val,

b) H-X-R^1-Ala-Y^1-Glu-Thr-Gly-Ala-Thr-Asn-Pro-Leu-R^2-X-Z, wherein Y^1 is Ala or Val,

c) H-X-R^1-Ala-Ala-Glu-Thr-Gly-His-Thr-Ser-Y^1-Val-R^2-X-Z, wherein Y^1 is Ser or Asn,

d) H-X-R^1-Y^1-Asp-Thr-Gly-His-Gly-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ala or Thr and Y^2 is Val or Ile,

e) H-X-R^1-Thr-Asp-Y^1-Gly-His-Asp-Thr-Val-Y^2-R^2-X-Z, wherein Y^1 is Ser or Thr and Y^2 is Ile or Val, and

f) H-X-R^1-Ala-Glu-Thr-Gln-Y^1-Gly-Thr-Y^2-Val-R^2-X-Z, wherein Y^1 is His or Tyr and Y^2 is Ile or Thr,

in which one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating amino acid sequence, R^1 and R^2 represent optional amino acid residues, wherein R^1 and R^2 together represent at most 25 amino acid residues, and Z represents $-NH_2$ or -OH, and antigenic parts thereof, together with a non-toxic pharmaceutically acceptable carrier and/or diluent.

10. A vaccine composition according to claim 9, c h a r a c t e r i s e d in that it comprises said picorna- and/or flavivirus antigen or antigens in an amount effective to protect a subject from diseases caused by picornaviruses and/or flaviviruses.

11. A vaccine composition according to claim 9, c h a r a c t e r i s e d in that it further comprises an antigen adjuvant in an amount which together with an amount of said picornavirus and/or flavivirus antigen or is effective to protect a subject from diseases caused by picornaviruses and/or flaviviruses.

Fig 1

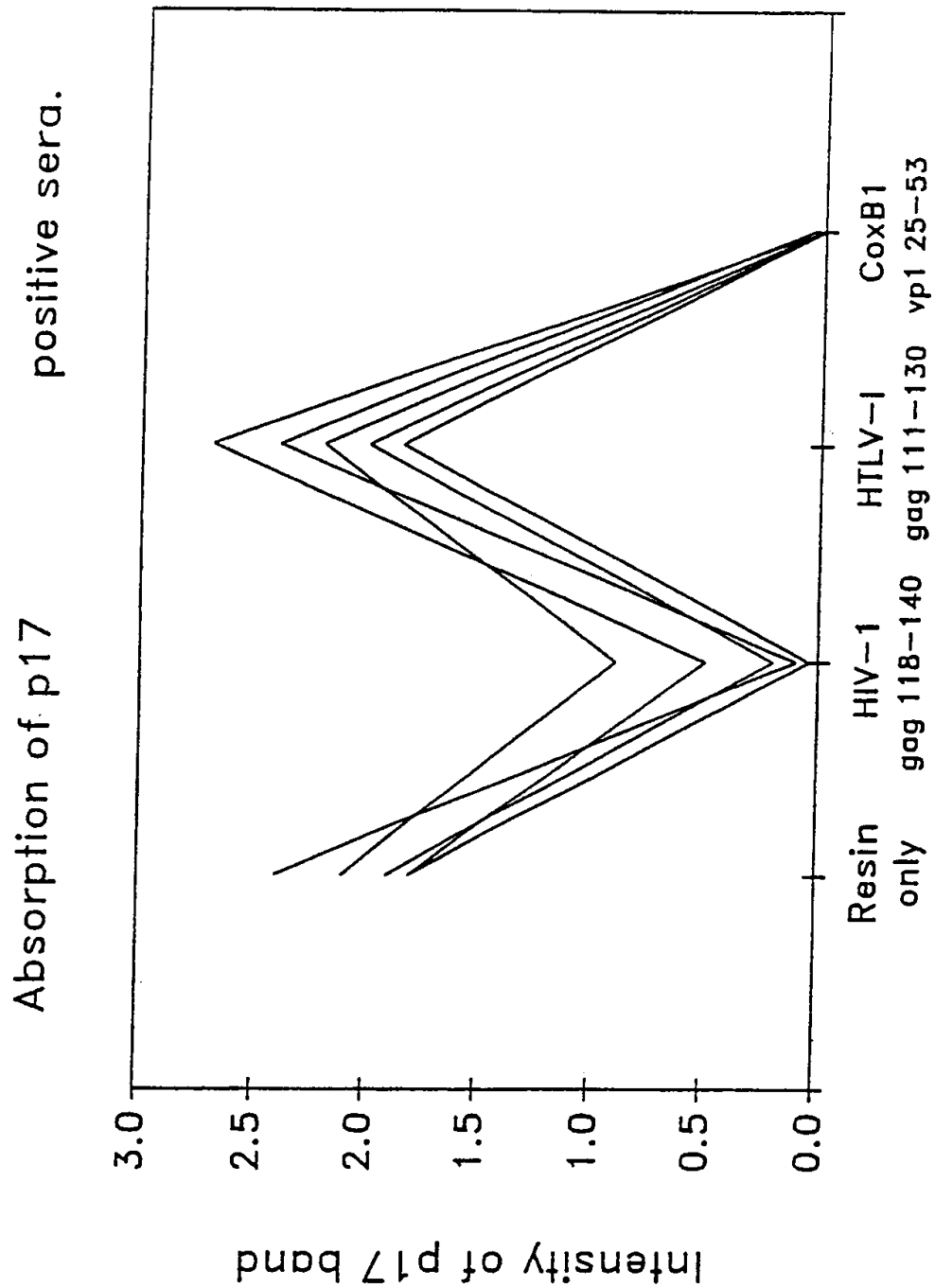
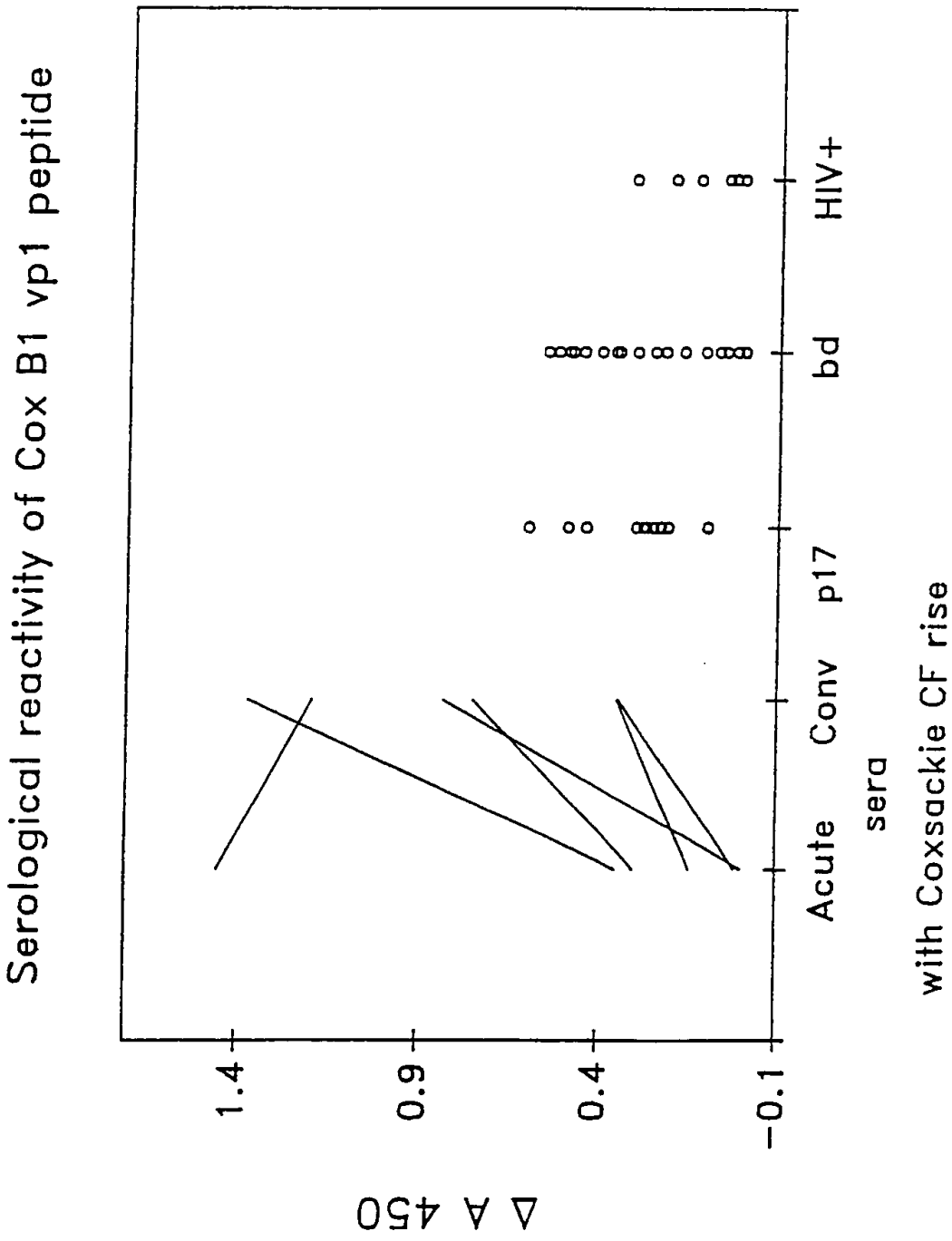

Absorption of p17
positive sera.
Intensity of p17 band
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Resin only
HIV-1 gag 118-140
HTLV-I gag 111-130
CoxB1 vp1 25-53

Fig 2


Serological reactivity of Cox B1 vp1 peptide
Δ A 450
1.4
0.9
0.4
-0.1
Acute Conv p17 bd HIV+
sera
with Coxsackie CF rise

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00542

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: C 07 K 7/08, A 61 K 39/125, 39/12, G 01 N 33/569

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K; C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Journal of Virology, Vol. 64, No. 5, May 1990 C E Fricks et al.: "Cell-Induced Conformational Change in Poliovirus:Externalization of the Amino Terminus of VPI Is Responsible for Liposome Binding", see page 1934 - page 1945 see page 1944, last paragraph	1B,2,3, 4B,6
Y	--	1B,2,3, 4B,6,9B, 10,11
P,X	Virology, Vol. 180, 1991 M Roivainen et al.: "Antigenic Regions of Poliovirus Type 3/Sabin Capsid ProteinsRecognized by Human Sera in the Peptide Scanning Technique", see page 99 - page 107 see in particular page 100 (peptides 13 and 14)	1B,2,3, 4B,6
	--	

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27th January 1992	1992 -01- 30
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Carl Olof Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with Indication, where appropriate, of the relevant passages	Relevant to Claim No
X	EP, A2, 0358485 (THE WELLCOME FOUNDATION LIMITED) 14 March 1990, see page 3, line 25; page 358 - page 4, line 4 example 1 peptide 526 and claims	1C,2,3, 4C,6
Y		1C,2,3, 4C,6,7, 9C,10, 11
	--	
A	Journal of Virology, Vol. 64, No. 2, 1990 M Roivainen et al.: "Improved distribution of Antigenic Site Specificity of Poliovirus-Neutralizing Antibodies Induced by a Protease-Cleaved Immunogen in Mice", see page 559 - page 562	1
	--	
Y	Advances in veterinary science and comparative medicine, Vol. 33, 1989 J M Hogle et al.: "Poliovirus: Three-dimensional Structure of a Viral Antigen", see page 65 - page 91 see in particular pages 72-73 and 80-81	1BC,2,3, 4BC,6,7, 9BC,10, 11
	--	
A	Proc.Natl.Acad.Sci., Vol. 82, February 1985 M. Chow et al.: "Synthetic peptides from four seprate regions of the poliovirus type 1 capsid protein VP1 induce netutralizing antibodies", see page 910 - page 914	1A-1C,2, 9-11
	--	
A	Nature, Vol. 317, September 1985 M G Rossmann et al.: "Structure of a human common cold virus and functional relationship to other picornaviruses", see page 145 - page 153; figure 4	1-11
	--	
X,Y	Aids, Vol. 3, No. 12, 1989 R B Ferns et al.: "Epitope location of 13 anti-gag HIV-1 monoclonal antibodies using oligopeptides and their cross reactivity with HIV-2", see page 829 - page 834 see table 3	3,5-8
	--	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X,Y	Chemical Abstracts, volume 113, no. 19, 5 November 1990, (Columbus, Ohio, US), Hinkula J et al.: "Epitope mapping of the HIV-1 gag region with monoclonal antibodies ", see pages-519, abstract 169943x, & Mol.Immunol. 1990, 27(5), 395- 403 --	3,5-8
X,Y	Journal of Virology, Vol. 63, No. 8, August 1989 M Niedrig et al.: "Epitope Mapping of Monoclonal Antibodies against Human Immunodeficiency Virus Type 1 Structural Proteins by Using Peptides", see page 3525 - page 3528 see table 1 --	3,5-8
A	JOURNAL OF ACQUIRED IMMUNE DEFICIENCY SYNDROMES, Vol. 4, 1989 B. WAHREN, ET AL.: "HIV-1 PEPTIDES INDUCE A PROLIFERATIVE RESPONSE IN LYMPHOCYTES FROM INFECTED PERSONS", see page 448 - page 456 SEE PAGE 449, PEPTIDE 30, TABLE 2, PEPTIDES 235-237 --	3,5-8
X,Y	IMMUNOLOGY, Vol. 67, 1989 T. MATHIESEN ET AL.: "MAPPING OF IGG SUBCLASS AND T-CELL EPITOPES ON HIV PROTEINS BY SYNTHETIC PEPTIDES", see page 453 - page 459 SEE PAGE 455, PEPTIDES 29 AND 30 --	3,5-8
X,Y	WO, A1, 8606414 (GENETIC SYSTEMS CORPORATION) 6 November 1986, SEE PEPTIDE 71, PAGES 9 AND 31 --	3,5-8
A	WO, A1, 8803032 (FOURNIER, MAURIELLE, J.) 5 May 1988, SEE THE WHOLE DOCUMENT AND IN PARTICULAR P 3,4,20-29 AND CLAIMS --	1D,2,3, 4,6,7, 8D,9, 10
A	US, A, 4810492 (FUJITA ET AL) 7 March 1989, see the whole document --	1-10

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	GB, A, 2209764 (NIPPON ZEON CO LTD) 24 May 1989, see the whole document --	1-10
A	DIALOG INFORMATION SERVICES, FILE 155, MEDLINE 66-91,DIALOG ACCESSION NO. 06323973, SRIVASTAVA AK ET AL: "ANTIGENICITY OF JAPANESE ENCEPHALITIS VIRUS ENVELOPE GLYCOPROTEINV3 (E) AND ITS CYANOGEN BROMIDE CLEAVED FRAGMENTS EXAMINED BY MONOCLONAL ANTIBODIES AND WESTERN BLOTTING", ARCH VIROL 1987, 96 (1-2) P 97-107 --	1
Y	JOURNAL OF VIROLOGY, Vol. 63, No. 2, 1989 C W MANDL ET AL: "ANTIGENIC STRUCTURE OF THE FLAVIVIRUS ENVELOPE PROTEIN E AT THE MOLECULAR LEVEL, USING TICK-BORNE ENCEPHALITIS VIRUS AS A MODEL", SEE FIG 1 AND TABLES 2 AND 3, FRAGMENT IRF1 --	1E,3,4E, 6-8,9E-11
A	INFECTION AND IMMUNITY, Vol. 37, No. 3, 1982 Franz X. Heinz et al: "Monoclonal Antibodies to the Structural Glycoprotein of Tick-Borne Encephalitis Virus", see page 869 - page 874 --	1E,4E, 9E
A	Dialog Information Services, File 155, Medline 83-91 Dialog accession no. 04796728, Heinz FX et al: Monoclonal antibodies to the structural glycoprotein of tick-borne encephalitis virus", Infect Immun Sep 1982, 37 (3) p 869-74 --	1-10
A	EP, A2, 0106837 (IMMUNO AKTIENGESELLSCHAFT FÜR CHEMISCH-MEDIZINISCHE PRODUKTE) 25 April 1984, see in particular pages 13-14 and fig I --	1-10
A	EP, A1, 0284791 (IMMUNO AKTIENGESELLSCHAFT FÜR CHEMISCH-MEDIZINISCHE PRODUKTE) 5 October 1988, see page 11; claims 31-42 --	1-10

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	AM.J.TROP.MED.HYG., Vol. 40, No. 6, 1989 B L Innis et al: "Identification of continuous epitopes of the envelope glycoprotein of dengue type 2 virus", see page 676 - page 687 see fig 1, hexapeptides 310-330 and Table 2, domain 17 --	1F,3,4F, 6-8,9F-11
A	ARCH VIROL, Vol. 105, 1989 J.G. Aaskov et al: "Serologically defined linear epitopes in the envelope protein of dengue 2", see page 209 - page 221; figure 1 --	
A	Dialog Information Services, File 155, Medline 66-91, Dialog accession no.07059248, Kurane I et al: "Dengue virus-specific human T cell clones. Serotype crossreactive proliferation, interferon gamma production, and cytotoxic activity", J Exp Med Sep 1 1989, 170 (3) p 763-75 --	1-10
A	Dialog Information Services, File 155, Medline 66-91 Dialog accession no. 06304270, Hall RA et al: "An enzyme immunoassay to detect Australian flaviviruses and indentify the encephalitic subgroup using monoclonal antibodies", Immunol Cell Biol Feb 1987, 65 (Pt 1) p 103-10 --	4-8
X	VIROLOGY, Vol. 177, 1990 J T Roehrig et al: "Antibodies to Dengue 2 Virus E-Glycoprotein Synthetic Peptides Identify Antigenic Conformation", see page 668 - page 675 see peptide 07 in tables 1 and 3 --	1F,3,4F, 6-8,9F-11
Y	VIROLOGY, Vol. 174, 1990 A G Pletnev et al: "Nucleotide Sequence of the Genome and Complete Amino Acid Sequence of the Polyprotein of Tick-Borne Encephalitis Virus", see page 250 - page 263; figure 4 --	1E,4E, 9E

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See next sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional seach fees.

Lack of unity a posteriori.

The general problem underlying the invention is not novel and a solution to it has already been found or does not involve an inventive step having regard to the state of the art as illustrated by:

a) EP, A2, 358 485

b) J Virology vol 64, no 5, May 1990, p 1934-45 (see page 1944, last paragraph)

As is evident from these documents the epitope in the region around aa 37-47 is known and is of interest for vaccine purposes and as a diagnostic antigen.

Therefore the original single general inventive concept is not acceptable anymore, making it necessary to reconsider the technical relationship between different solutions mentioned.

This leads to their regrouping under distinct subjects as listed below, each subject now falling under its own inventive concept.

1. Claims 1a, 2, 3, 4a, 6-8, 9a, 10-11: Peptides...AETGHTSQV....from Coxsackie viruses and their application in diagnostics or in vaccines.

2. Claims 1b, 2, 3, 4b, 6-8, 9b, 10-11: Peptides....AAETGATNPL....from Polio virus and their application in diagnostics or in vaccines.

3. Claims 1c, 2, 3, 4c, 6-8, 9c, 10-11: Peptides....AAETGHTS.V....from Rhinovirus and their application in diagnostics or in vaccines.

4. Claims 1d-f, 2, 4d-f, 6-8, 9d-f, 10-11: Peptides....ADTGHGTV..., ...T...TD.GHDTV... and AETQ.GT.V... from Flaviviruses and their application in diagnostics or in vaccines.

5. Claims 3, 5: Diagnostisc antigen with affinity for HIV-1 p17 antibodies and use therof in discriminative assays for HIV positive serum samples.

One search fee however was estimated to cover search for all picorna viruses.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00542

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 30/11/91
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A2- 0358485	90-03-14	AU-D-	4122689	90-03-15
		JP-A-	2223594	90-09-05
WO-A1- 8606414	86-11-06	AU-B-	571128	88-03-31
		AU-B-	597884	90-06-14
		AU-D-	5572786	86-10-16
		AU-D-	5773386	86-11-18
		EP-A-	0201716	86-11-20
		EP-A-	0220273	87-05-06
		JP-T-	62502617	87-10-08
		US-A-	4629783	86-12-16
		US-A-	4768607	88-09-06
WO-A1- 8803032	88-05-05	JP-T-	1501203	89-04-27
US-A- 4810492	89-03-07	BE-A-	905815	87-03-16
		CA-A-	1270780	90-06-26
		DE-A-C-	3641040	87-12-10
		FR-A-	2599626	87-12-11
		GB-A-B-	2191201	87-12-09
		JP-A-	62286930	87-12-12
		NL-A-	8603017	88-01-04
GB-A- 2209764	89-05-24	FR-A-	2620459	89-03-17
		JP-A-	1074982	89-03-20
		US-A-	5021347	91-06-04
EP-A2- 0106837	84-04-25	AT-A-B-	384363	87-11-10
EP-A1- 0284791	88-10-05	JP-A-	64002586	89-01-06